CYTOKINE AND MATRIX METALLOPROTEINASE EXPRESSION IN PIGMENTED VILLONODULAR SYNOVITIS MAY MEDIATE BONE AND CARTILAGE DESTRUCTION

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ABSTRACT

Background: Pigmented villonodular synovitis (PVNS) is characterized by hypervascular proliferative synovium containing multinucleated giant cells, macrophages, and hemosiderin. The destruction of articular cartilage and erosion of periarticular bone is thought to be mediated by matrix metalloproteinases (MMPs). Expression of MMPs in some tumors appears to be stimulated through local production of cytokines, and several specific cytokines (TNFα, IL-1, and IL-6) play an important role in the stimulation of osteoclastic bone resorption. The role of cytokine secretion and regulation of MMP production in PVNS has not been investigated.

Design: In the present study, ten specimens from eight patients (ages 19 to 80) were evaluated histologically according to a modified Mirra classification and immunohistochemically (IHC) for the expression of MMP-9 (92 kDa gelatinase B), tumor necrosis factor alpha (TNFα), interleukin 1-ß (IL-1ß), and interleukin 6 (IL-6). Localization of IL-6 and TNFα production was confirmed by in situ hybridization (ISH) for mRNA.

Results: All specimens, regardless of location (six knees, one ankle, one subtalar joint), showed diffuse and intense immunoreactivity for cytokines in the giant cells and synovial cells, and less intense and diffuse staining in the activated macrophages. Staining in the fibroblastic elements was minimal. In situ hybridization for TNFα and IL-6 mRNA mirrored the immunohistochemistry results, although the IL-6 staining was weaker than that for TNFα. Immunoreactivity for MMP-9 was diffuse and strong in giant cells, diffuse and moderate in synovial cells, and focal and moderate to strong in macrophages. In contrast, normal synovium demonstrated focal, moderate immunoreactivity for IL-1ß, IL-6, TNFα and MMP-9 localized in the synovial lining cells.

Conclusion: These findings suggest that periarticular bone resorption and cartilage destruction which characterize PVNS may be related to the expression of inflammatory cytokines, which in turn stimulate MMP production.

INTRODUCTION

Pigmented villonodular synovitis (PVNS) is a slowly progressive monoarticular arthropathy characterized by an exuberant proliferation of synovium. The synovial tissue is mainly composed of mononucleated stromal cells and multinucleated giant cells embedded in a loose fibrous matrix that contains a high content of hemosiderin. These tumors can destroy cartilage and frequently cause periarticular bone resorption. In fact, a highly aggressive pattern of bone resorption is characteristic of these tumors and distinguishes them from other inflammatory arthropathies.

PVNS typically occurs in the fourth and fifth decades of life. PVNS presents with two patterns of growth; in one pattern, PVNS is localized to a specific region of the joint, while in the other, the tumor diffusely involves the entire synovial surface of the joint. Treatment consists of surgical excision, which may involve complete synovectomy for the diffuse form of this disease. Following excision, PVNS has a recurrence rate of approximately 20 to 25%, and both adjuvant external beam irradiation and intraarticular injection with yttrium 90 have been advocated for control of the tumor.

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Despite treatment, a certain percentage of cases result in extensive bone and joint destruction and ultimately require joint replacement or fusion. At this time, little is known about the pathophysiology of the bone and cartilage destruction. It has been shown that both the stromal cells and multinucleated giant cells of PVNS express parathyroid hormone related peptide (PTHrP), as well as the PTH/PTHrP receptors, a factor which has been shown to stimulate osteoclast formation and activity and has been implicated in pathologic bone destruction from a variety of causes[12]. More recently, it has been shown that the multinucleated giant cells of PVNS, and the closely related giant cell tumor of tendon sheath, express tartrate-resistant acid phosphatase, calcitonin receptors, and the $a_v\beta_3$ integrin (vitronectin receptor), all of which are characteristic of osteoclasts[4]. Studies have shown that matrix metalloproteinases (MMPs), collagenase, and stromelysin are produced by cells in PVNS. However, synovial lining cells are the primary source of MMPs. Occasional infiltrating mononucleated histiocytic cells also produce the mRNA of MMPs. The giant cells of PVNS did not produce mRNA for either collagenase or stromelysin[3].

The current investigation was conducted to further characterize the catabolic activity of PVNS. Since the cytokines IL-1, IL-6, and TNFα have been associated with both bone resorption and cartilage catabolism, the expression of these cytokines was examined by immunohistochemistry and in situ hybridization. Additionally, the expression of MMP-9 (gelatinase B), which typically is highly expressed in osteoclasts, was examined.

METHODS

Tissue Specimens

Tissue samples of PVNS were retrieved from the surgical pathology files at our institution. Synovium involved by rheumatoid arthritis (in which synovial lining cells are known to express IL-1β, IL-6, TNFα and MMPs) served as the positive control for detection of these cytokines. Tissue from all cases was fixed in formaline prior to routine paraffin embedding, sectioning and staining with hematoxylin and eosin. Additional five micron sections for immunohistochemistry and in situ hybridization were cut and mounted on poly-L-lysine coated (PLL) slides.

Immunohistochemistry (IHC)

Immunohistochemistry was performed by the biotin-streptavidin method. The primary antibodies used in this study are listed in Table 1. Endogenous peroxidase activity was consumed with hydrogen peroxide (H_2O_2, 3%) and sections were rinsed in phosphate buffered saline (PBS). The sections were incubated with the primary antibody overnight at 4° C, using the dilutions shown in Table 1. A 0.5% solution of PBS-bovine serum albumin was used as the diluent for the antibodies and was applied alone as the negative control. The sections were incubated with secondary antibodies (Vector Laboratories, Burlingame, CA), used at 1:200 dilution, at room temperature for 30 minutes. Horseradish peroxidase-streptavidin conjugate (Jackson Immunoresearch Labs, Inc., West Grove, PA), used at 1:1000 dilution, was applied and allowed to incubate for 30 minutes. The chromagen, 3-amino-9-ethylcarbazole (Zymed Laboratories Inc., South San Francisco, CA), was applied to each section. Sections were then counterstained with Mayer's hematoxylin and mounted with permanent aqueous media.

TABLE 1
Antibodies Used for Immunohistochemistry

Primary Antibody	Dilution	Source
IL-1β	1:100	Genzyme (Cambridge, MA)
IL-6	1:40	Genzyme (Cambridge, MA)
TNFα	1:1000	Genzyme (Cambridge, MA)
MMP-9	1:100	polyclonal to synthetic MMP-9 peptide (NCI, Dr. Stetler-Stevenson)

Inflammatory cells in tissue sections of rheumatoid synovium, which served as a positive control, demonstrated strong reactivity for all three cytokines and MMP-9 in synoviocytes and inflammatory cells. In negative controls, in which the primary antibody was omitted, no staining occurred.

In Situ Hybridization (ISH)

In situ hybridization was performed using a modified digoxigenin-11-dUTP labeled non-radioactive technique (Genius™ kit, Boehringer-Mannheim, Indianapolis, IN) with immunodetection using an anti-digoxigenin antibody, according to our previously published procedure[2]. A 30 base oligonucleotide probe complementary to IL-6 mRNA, a 454 base cDNA probe complementary to TNFα mRNA, and a 25 base control nonsense probe (chick aggrecan with no known human homologies) were used in this study. The tissue sections were deproteinized with HCl and digested with proteinase K. The sections were further digested with hyaluronidase and then fixed in 4% paraformaldehyde. Sections were rinsed in PBS and acetylated to reduce background

TABLE 2
PVNS Cases

CASE	AGE, SEX	SITE
1-a	19, M	R Knee
-b		
2	80, F	L Knee
3	31, F	R Ankle
4-a	71, F	L Knee
-b		
5	31, M	R Knee
6	56, F	R Knee
7	28, F	L Knee
8	39, F	R Midfoot

staining. The specimens were incubated with prehybridization solution for one hour and rinsed in saline-sodium citrate (SSC). The digoxigenin-11-dUTP labeled oligonucleotide was applied and the specimens were incubated overnight at 37°C. The slides, which underwent post-hybridization stringency washes in decreasing concentrations of SSC, were then rinsed in buffer 1 (tris-HCl, NaCl, pH 7.5) and preblocked in normal sheep serum. The excess was decanted and the alkaline phophatase conjugated antibody to the labeled probes was added. The slides were then washed in buffer 1(tris-HCl, NaCl, pH 7.5), and freshly prepared color solution (Nitro Blue tetrazolium) was added to each section. The reaction was incubated for ten to 12 hours in a light-tight box. The color reaction was stopped and the specimens were then dehydrated, rinsed in xylene, and prepared for microscopic examination.

Histologic Grading of IHC and ISH

Sections were evaluated semi-quantitatively for distribution and intensity of staining. Distribution was graded as focal (<10% of cells), moderate (10-50%) or diffuse (>50%). Intensity was graded as negative (0), weak (+1), intermediate (+2) or strong (+3). The product of the scores for the intensity and the distribution of cells (focal=1, moderate=2, diffuse=3) was divided by three to determine the overall immunostaining score, shown as the number of plus signs in Table 5. Thus the immunostaining score takes into account both the intensity of the staining and the percentage of cells which stain. Inflammatory cells in rheumatoid synovium demonstrated the well described strong reactivity for all three cytokines[15,16] and served as a positive control. Negative controls incubated in the absence of primary antibody showed no staining.

RESULTS

Patient Demographics

Histologic specimens of PVNS were examined from eight patients, including both original and recurrent lesions from two patients, for a total of ten specimens (Table 2). The tumors occurred in patients with a wide age range (19 to 80 years), with a mean of 44 years. The lesions involved the knee in eight cases (six patients and both recurrent tumors) and the foot and ankle in two cases. The tumor was variable in the extent of synovial involvement among the patients, although the two recurrent tumors demonstrated diffuse and extensive involvement of the synovium. Plain radiographs of the involved joints demonstrated erosive changes in the periarticular bone in most of the cases, a finding also demonstrated by magnetic resonance (MR) imaging in several cases. The tumors were treated in all cases by partial or total synovectomy, depending on the extent of involvement. The two recurrent cases were successfully treated by repeat surgical synovectomy.

Histologic Findings

The tumors consisted of a synovial proliferation containing an abundance of fibroblastic stromal cells, giant cells, xanthomatous or histiocytic stromal cells, and hemosiderin. In addition, the intraarticular surfaces of the villonodular tissue demonstrated lining cells resembling normal synoviocytes. The cellular components were evaluated semi-quantitatively using a modified grading system of Mirra proposed for characterization of aggressive membranes in implant-related osteolysis (Table 3)[5,11]. The results of the analysis of our cases are presented in Table 4. The proportion of the various cellular components differed, demonstrating heterogeneity among the tumor specimens. However, contrary to our initial expectations, the cellular makeup did not correlate with the clinical aggressiveness of the tumors in terms of radiographic evidence of bone and cartilage destruction or association with recurrence. Furthermore, there was no correlation between the radiographic aggressiveness and the cytokine or MMP-9 expression.

The interface between the bone and the tumors exhibited multiple resorption lacunae with associated osteoclast-like giant cells. The osteoclast-like giant cells blended into the surrounding stroma and were indistinguishable from giant cells located within the tumors.

TABLE 3
Definition of Criteria for Modified Mirra Grading System for Morphologic Evaluation of PVNS

CRITERIA SCORE

		1	2	3	4
CELL TYPE	**HISTIOCYTIC CELLS**	focal parasinusoidal aggregates	extensive parasinusoidal aggregates	parasinusoidal aggregates, small confluent areas	replacement of architecture by sheets
	HEMOSIDERIN	<10 particles per mpf	10-20 particles per mpf	21-50 particles per mpf	>50 particles per mpf
	GIANT CELL	1-2 / mpf	3-8 / mpf	>8 / mpf	
	FIBROBLASTS	focal increase	large aggregates		

mpf = medium power field (200x)

TABLE 4
Histological Analysis of PVNS Cases Based on Modified Mirra Grading System

Case	GIANT	HEMOSID	HISTIO	FIBRO
1 - a	D:3	F:1	1	0
- b	M:3	F:3	3	2
2	D:1,M:2	M:2	3	2
3	M:1	M:1	4	2
4 - a	D:3	M:2	0	0
-b	D:3	D:4	1	2
5	M:1,F:2	M:4	2	1
6	F:1	D:2	3	0
7	M:1	M:2,F:3	2	1
8	M:3	F:4	4	2

D= diffuse, >50% of tissue; M=moderate, 10-50% of tissue; F=focal, <10% of tissue

TABLE 5
Immunohistochemical Analysis of PVNS for Cytokine and MMP-9 Expression

CASE	TNFα	IL6	IL1	MMP9	Cells Expressing
1-a	+	+	+	++	S > H > G
-b	++	++	++	+++	G,H > F
2	+++	+++	+++	++	G,S > H,F
3	++	+++	++	++	S > G > H
4-a	++	+	++	++	G > S > H
-b	++	++	++	++	G > S,H > F
5	+++	+++	+++	+++	G,S > F,H
6	++	+	+++	+++	H,S > G
7	+++	+++	+++	++	G > S > F
8	+++	++	++	++	G,S > H

S = Synovial cells; G = giant cells; F = fibroblasts; H = histiocytic cells

Immunohistochemical Findings

All of the tumor specimens demonstrated diffuse immunoreactivity for the cytokines IL-1ß, IL-6, and TNFα based on the overall immunostaining score (Table 5). The intensity of the staining varied somewhat among the tumors and among the individual cell types within each particular tumor. In general, the giant cells and the synovial lining cells stained most intensely, the xanthomatous stromal cells (histiocytes) were intermediate, and the fibroblastic stromal cells least intense (Table 5). The pattern and intensity of staining was similar for IL-1ß, IL-6, and TNFα (Figures 1A-C).

Immunoreactivity for MMP-9 was also examined in each of the tumors. MMP-9 expression was similar to that of the cytokines, generally diffuse and strong in giant cells and more focal, but with intermediate to strong intensity in histiocytes (Figures 2A and B). Fibroblastic stromal cells showed the least intensity. Some weak expression was also seen in scattered cells on the surface layer of uninvolved areas of normal synovium.

In Situ Hybridization Findings

In situ hybridization was performed with oligonucleotide probes specific for TNFα and IL-6. TNFα mRNA expression was similar to that observed in the immunohistochemical stains. Strong signal was present in giant cells and histiocytic stromal cells, with less intense staining of the fibroblastic cells. The IL-6 signal was similar in distribution to TNFα in the sections, but of lesser intensity. Background staining, using a probe for chick aggrecan with no known human homologies, was negligible (Figures 3A-C). The ISH results are summarized in Table 6.

TABLE 6
In Situ Hybridization of PVNS Specimens for IL-6 and TNFα

CASE	TNFα	IL6
1-a	+	±
-b	+++	++
2	++	++
3	+++	++
4-a	+++	+
-b	+++	+
5	+++	++
6	++	++
7	+++	++
8	+++	+

(Staining intensity scale: ± to +++)

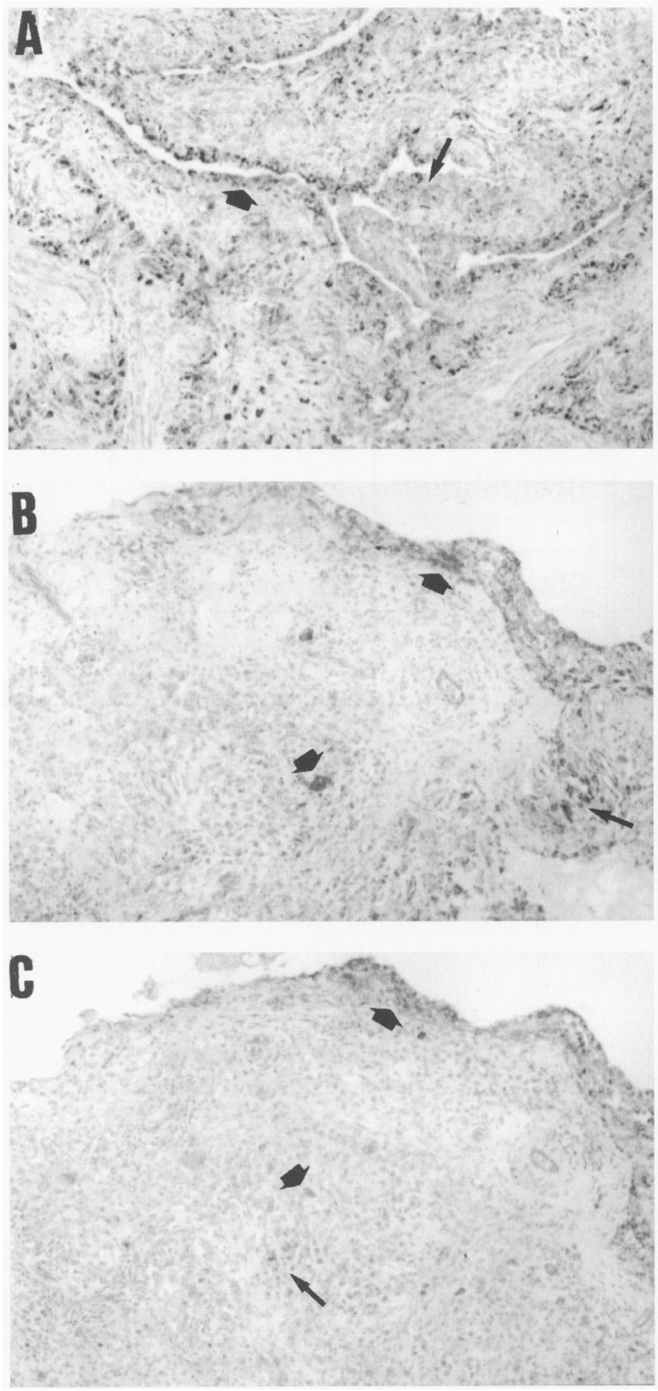

Figure 1. Immunostaining from Case 5. Fig. 1A: TNFα immunostaining showing more intense staining in synovial lining cells (thick arrow) with less intense staining of histiocytes (thin arrow). Fig. 1B and 1C: IL-1 immunostaining (B) and IL-6 immunostaining (C) also show more intense staining of synovial lining cells and giant cells (thick arrows) and less intense staining of histiocytes (thin arrow). Original magnification = 100x.

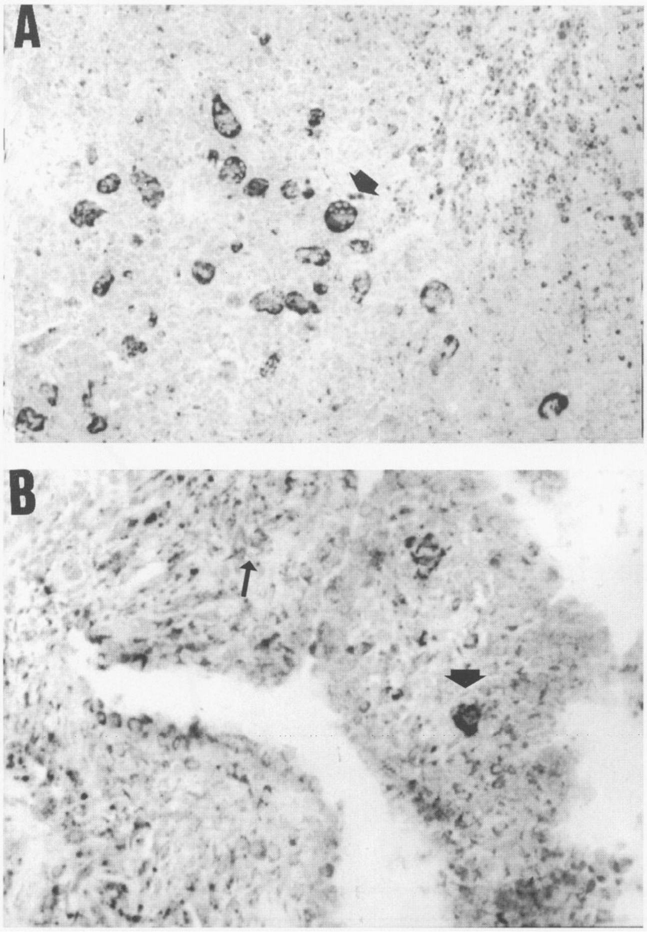

Figure 2. Immunohistochemistry for MMP-9. Figure 2A: Intense staining of giant cells in case 4-b. Original magnification = 100x. Fig. 2B: Staining of giant cells (thick arrow) and less staining of scattered histiocytes (thin arrow) in case 5. Original magnification = 200x.

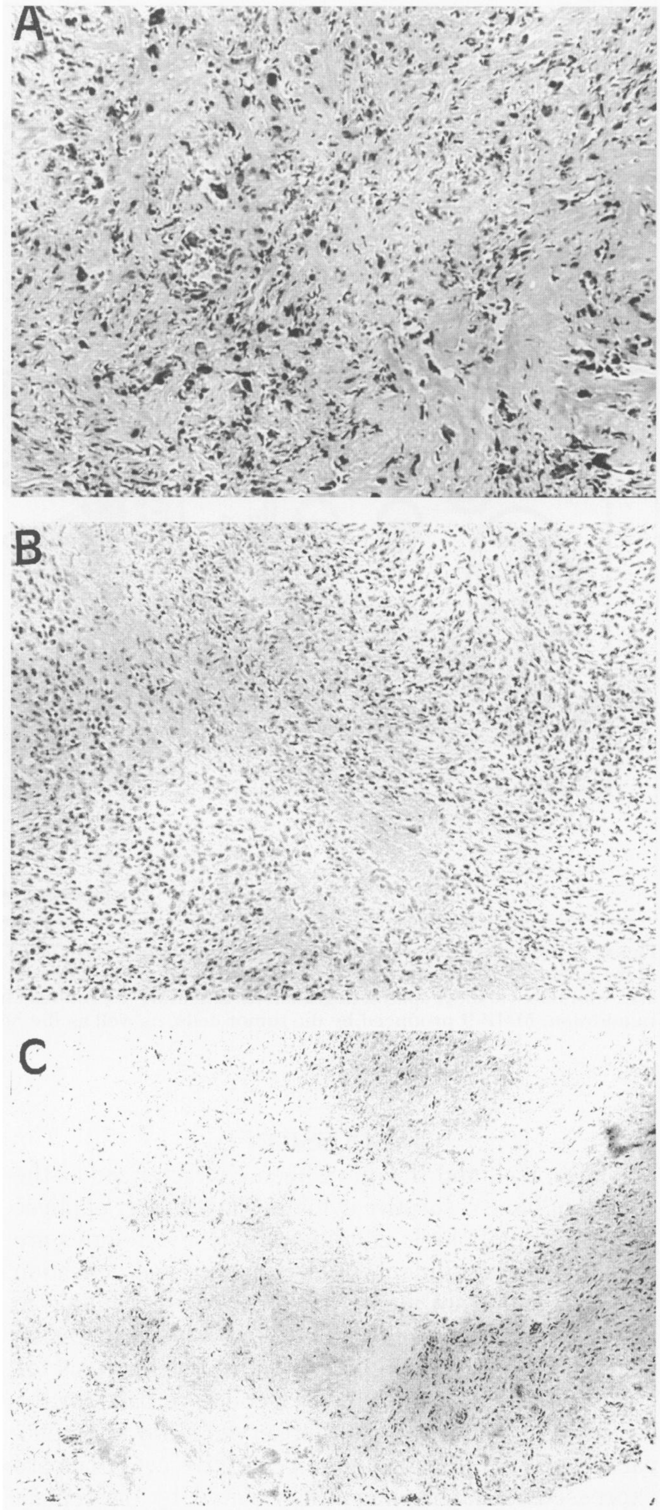

Figure 3. In situ hybridization in Case 8. Fig. 3A: TNFα, with intense (+++) signal in giant cells and histiocytes. Fig. 3B: IL-6 with diffuse, low-level (+) signal in histiocytic stromal cells. Fig. 3C: Nonsense cDNA oligonucleotide probe with minimal background. Original magnification = 100x.

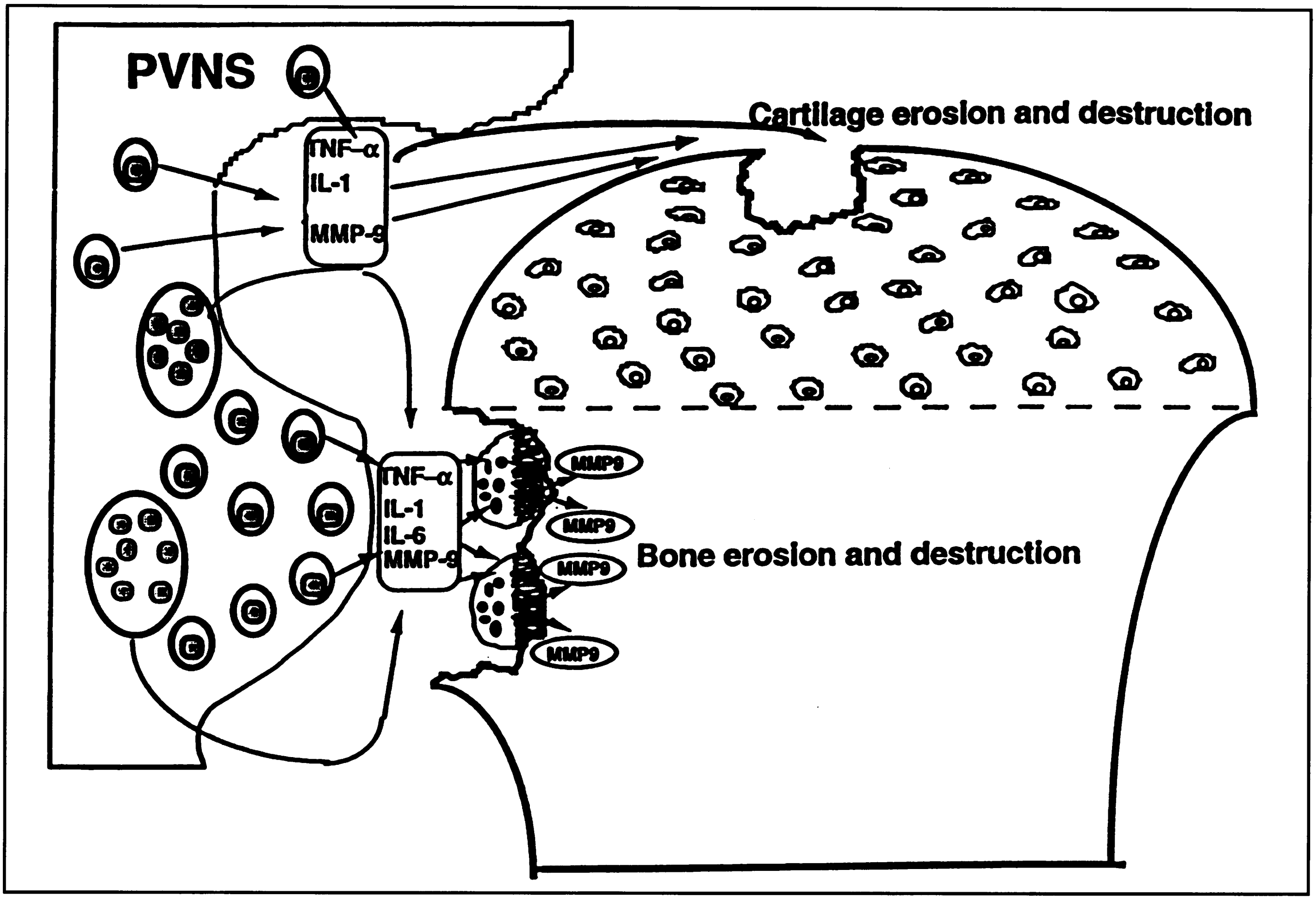

Figure 4. Synovial involvement with PVNS leads to secretion of TNFα ,IL-6, and IL-1 by the giant cells, synovial cells, and histiocytic cells of the tumor. These bone resorptive cytokines stimulate osteoclast formation and activation, resulting in bone destruction. TNFα and IL-1 also stimulate cartilage matrix degradation, and MMP-9 produced by the tumor cells may participate directly in cartilage matrix breakdown. In addition, MMP-9 produced by the tumor cells, as well as the host osteoclasts, may participate in bone resorption.

DISCUSSION

TNFα, IL-1, and IL-6 have been characterized as the dominant bone resorptive cytokines in a variety of physiologic and pathologic conditions. These cytokines are involved in recruitment, differentiation, and activation of osteoclasts from monocytic precursors, resulting in stimulation of bone resorption[13]. Increased expression of IL-6 in bone has been observed in osteoporosis following estrogen loss and may account, in part, for the accelerated rate of bone loss observed following menopause[1,8]. All three of the bone resorptive cytokines are expressed in macrophages stimulated by particulate wear debris and have been implicated in bone resorption associated with loosening of orthopaedic implants[7]. The overexpression of the cytokines stimulates osteoclastic bone resorption causing the osteolysis and implant loosening. In addition, these cytokines are also expressed in osteoclasts and may function by autocrine mechanisms to enhance osteoclastic activity[9].

PVNS is frequently characterized by periarticular erosions of the involved synovium as well as focal periarticular "cystic" appearing lytic lesions. Histologic examination of these lesions usually demonstrates infiltration of the tumor tissue into the bone in these areas. The ingress of the neoplastic tissue into focal intraosseous lytic lesions is often better visualized by MR imaging than by plain radiographs. MR imaging typically demonstrates low signal intensity on all imaging sequences in areas with PVNS due to the iron signal from the high hemosiderin content. The histologic examination of the lytic intraosseous lesions and periarticular erosions in the present study demonstrated typical PVNS tissue, with active osteoclastic resorption along the bone-tissue interface. The osteoclasts in the resorption lacunae were indistinguishable from those in the tumor tissue, and the immunohistochemical staining for resorptive cytokines and MMP-9 was identical to that of the giant cells in the tumor. The presence of resorptive cytokine expression, as well as MMP-9 expression, within these tumors suggests that the periarticular bone destruction characteristic of these lesions may be caused by increased expression of these factors. This is consistent with previous studies of the giant cells and histiocytic cells in PVNS which demonstrated that these cells express numerous characteristics of bone resorbing osteoclasts[4].

The immunohistochemical findings in the present study were further substantiated by in situ hybridization for TNFα and IL-6. Although the staining for IL-6 was substantially less than that observed for TNFα, the pattern of mRNA expression was similar for these two cytokines and mirrored the immunohistochemical pattern for protein expression. The greater expression of TNFα mRNA that was apparent is more likely a reflection of the use of a much longer cDNA probe for this cytokine. Quantitative comparisons between differing target mRNAs are not valid with these methods.

Normal articular cartilage does not express significant levels of IL-1, IL-6, and TNFα, but cytokine expression occurs as one of the early events during articular cartilage degeneration[6,14]. In cartilage, these cytokines are known to stimulate matrix metalloproteinase secretion and thereby contribute to degradation of the cartilaginous matrix. TNFα causes stimulation of synovial cells to secrete MMPs and has been implicated as a major contributor to cartilage and bone destruction in inflammatory arthritides such as rheumatoid arthritis[9]. IL-1 has also been demonstrated to stimulate cartilage matrix degradation[10]. Expression of MMP-9 in synovium and chondrocytes of degenerating cartilage may also play a role in enzymatic degradation of the cartilaginous matrix (R.Rosier, unpublished observations). Given that the consequence of untreated PVNS may be progressive cartilage destruction, the increased expression of MMP-9 may contribute directly to this process as well. Many other cytokines and proteases have been demonstrated in inflammatory processes and cartilage degeneration and, undoubtedly, other factors are expressed in PVNS as well.

In conclusion, the present study demonstrates expression of IL-1ß, IL-6, TNFα, and MMP-9 in the tumor tissue of all ten cases of PVNS analyzed by immunohistochemistry and in situ hybridization. The presence of these bone resorptive cytokines may explain the periarticular lytic lesions characteristic of this disease. These same cytokines are also known to stimulate cartilage matrix degradation and may play a role in mediating the progressive articular degeneration associated with PVNS. Osteoclasts express MMP-9 and this may play either a direct or indirect role in bone resorption. In addition, this enzyme could also directly contribute to cartilage matrix degradation. A summary of these interactions is presented diagramatically in Figure 4. This new information on PVNS may aid in the development of future strategies for pharmacologic modulation of cytokine expression, bone resorption, or MMP inhibition as potential alternatives or adjuvants to surgical treatment for the disease.

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